Direct diagnosis of MbTb complex in AIDS patients by the genetic technique TMA of the AMTD gen-probe system

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This paper has been announced in the 27th Annual Congress of the ESM 2006, London, England, 9-12 July 2006.

ABSTRACT: The purpose of the study was the evaluation of the Gen-Probe Amplified Mycobacterium Tuberculosis Direct test (AMTD) for the diagnosis of possible Tb infection to AIDS patients. During the years 2002-2005 we examined 57 biological specimens from 19 different adult patients suffering from active HIV infection, 3 of which mentioned a Tb infection in the past. From each patient studied, the following biological specimens were derived: a) sputum, b) bronchial aspirates and c) whole blood. All the specimens were examined by the following laboratory examinations: Gen-Probe, Ziehl-Neelsen (Z-N) staining and Lowenstein-Jensen culture (L-J).

14 biological specimens, from 7 patients, were Gen-Probe positive (>45000 RLU).

Conclusion: Gen-Probe is recommended to be applied to every patient with active HIV infection because it is a) the most sensitive (sensitivity 98%) and specific method in comparison to the conventional ones: Z-N (sensitivity 55%) and L-J (sensitivity 75%), b) the results are ready in only 3.5 hours. Thus, Gen-Probe leads to the quick and timely Tb diagnosis contributing i) to the prompt start of anti-Tb drug therapy and ii) to the reduction of the hospital nursing costs.

Key Words: Tuberculosis, Mycobacteria, AIDS patients, HIV infection, Probe.

INTRODUCTION

While AIDS has exploded over the last decade, TB has increased 20% rise and today TB kills one out of three AIDS patient worldwide.

The two diseases represent a deadly combination, since both are more destructive together than either is alone. HIV infection is the most potent risk factor for converting latent TB into active transmissible TB -accelerating the spread of the disease- while TB bacteria help accelerate the progress of the AIDS infection in the patient¹-². Today TB is the leading cause of death in persons who are HIV positive.

When someone with latent TB becomes co-infected with HIV, his/her risk of developing active TB increases by a factor of 30-50³⁴⁶. HIV-positive individuals do not have the internal immune-system resources to keep the mycobacterium TB in check. In fact, they are 30 times more likely to develop active TB than people who are HIV-negative. As a result, they succumb to the disease at an alarming rate. Although people with AIDS are dangerously susceptible to a number of opportunistic infections, TB is clearly the leading killer. The TB bacterium enhances HIV replication and might accelerate the natural progression of HIV infection⁸⁹.

For these reasons, TB poses a high level of risk for HIV patients to develop active tuberculosis, which will take one in three of their lives. In order to stem
the tide of deaths from AIDS, therefore, it is crucial to work towards new, faster-acting ways to combat TB and develop preventive TB treatment programs\cite{3,4}.

**MATERIAL AND METHODS**

The study was conducted to the Mycobacterium Tuberculosis (M\textit{b}Tb) Reference Center of N. Greece (covers a population of \approx 2500000 people). During the years 2002-2005 we examined 57 biological specimens from 19 different adult patients suffering from active HIV infection, 3 of which mentioned a Tb infection in the past. From each patient studied, the following biological specimens were derived: a) sputum, b) bronchial aspirates and c) whole blood. All the specimens were examined by the following laboratory examinations: Gen-Probe, Ziehl-Neelsen (Z-N) staining and Lowenstein–Jensen culture (L-J).

**RESULTS**

The AMTD results were interpreted according to the manufacturer’s recommendations. 14 biological specimens, from 7 patients, were Gen-Probe positive (>45000 RLU). In particular, 5 were of sputum, 7 were of bronchial aspirates and 2 were of whole blood. Table 1 shows the laboratory tests results for each specimen respectively. Two of these patients mentioned a Tb infection in the past. Our results were in agreement to the clinical examination’s and the chest X-ray’s findings.

**DISCUSSION**

TST is not fully reliable at detecting TB infections among HIV-infected people because their weakened immune systems often cannot mount a strong enough defence against the injected proteins to cause swelling.

Around the world, attempts are being made to improve collaboration between TB and HIV programmes. It is being proposed that everyone diagnosed with TB should be tested for HIV and vice-versa, and that treatment programmes should share facilities and expertise.

\textit{HIV services can be established in TB clinics by}\cite{7}:

- Persuading TB clinics to provide HIV education and voluntary counseling and testing (VCT) services.
- Helping TB control programs to provide HIV training and to develop guidelines for managing HIV-infected TB patients.

\textit{TB control activities can be incorporated within HIV services by}\cite{10}:
- Promoting and providing TB education and training wherever HIV services are delivered.
- Involving AIDS community-based care groups in TB treatment delivery.
- Providing TB preventive therapy to persons living with AIDS.

The probe detection methods are practiced for years in research laboratories, but their use has recently been broadened in clinical labs\textsuperscript{11,12,13,14}. The DNA detector, which is the special part of the procedure and in the same time the most sensitive one, is found in one copy in every cell. In continuance, the ribosomal ribonucleic acid (rRNA) was used in the more sensitive procedures of hybridism. As there are more copies of rRNA in the cell (2000 to 10000), the TMA method is more sensitive than the ones using DNA, which exists in only one copy (in the form of a double spiral chain) in every target cell. The genome RNA of bacteria is a molecule of single basis chain. It doesn’t have Thymidin and instead of that it has Uracil that can connect to Adenine and is the translator of genetic code. The RNA (single chain) is by nature more sensitive than the DNA and it is superior to the DNA because the danger of infection is reduced (possibility of carrying out the determination at the same place - easy disinfection by usual means).

The ribosomal RNA is found in alive and active cells, while the DNA is also found in dead. Moreover, the TMA technique of amplification and hybridism of specific genetic sequences is the solution for the lab detection of Tb, for economical, social and moral reasons.

**CONCLUSION**

Gen-Probe is recommended to be applied to every patient with active HIV infection because it is a) the most sensitive (sensitivity 98%) and specific method in comparison to the conventional ones: Z-N (sensitivity 55%) and L-J (sensitivity 75%), b) the results are ready in only 3.5 hours. Thus, Gen-Probe leads to the quick and timely Tb diagnosis contributing i) to the prompt start of anti-Tb drug therapy and ii) to the reduction of the hospital nursing costs.
ΠΕΡΙΛΗΨΗ: Σκοπός της μελέτης ήταν η εκτίμηση της γενετικής τεχνικής Amplified Mycobacterium Tuberculosis Direct test (AMTD) της Gen-Probe για τη διάγνωση πιθανής φυματικής λοίμωξης σε ασθενείς με AIDS. Κατά τη διάρκεια των ετών 2002-2005 εξετάσαμε 57 βιολογικά δείγματα από 19 διαφορετικούς ενήλικες ασθενείς πάσχοντες από ενεργό λοίμωξη με τον ύπο HIV, από τους οποίους οι 3 ανέφεραν φυματική λοίμωξη στα παρελθόντα. Από κάθε ασθενή που μελετήθηκε εφαρμόστηκαν τα ακόλουθα βιολογικά δείγματα: α) πτύελα, β) βρογχικές εκκρίσεις και γ) ολικό αίμα. Σε όλα τα βιολογικά δείγματα εφαρμόστηκαν οι παρακάτω εργαστηριακές εξετάσεις: Gen-Probe, χρώση Ziehl-Neelsen (Z-N) και καλλιέργεια σε θρεπτικό υλικό Lowenstein-Jensen (L-J). 14 βιολογικά δείγματα από 7 ασθενείς ήταν θετικά με Gen-Probe (>4500 RLU). Συμπέρασμα: Η Gen-Probe προτείνεται να εφαρμόζεται σε κάθε ασθενή με ενεργό HIV λοίμωξη επειδή: α) είναι η περισσότερο ευαίσθητη (ευαισθησία 98%) και ειδικά μέθοδος σε σύγκριση με τις παραδοσιακές μεθόδους: χρώση Z-N (ευαισθησία 55%) και καλλιέργεια σε υλικό L-J (ευαισθησία 75%), β) τα αποτελέσματα είναι έτοιμα μόνο σε 3,5 ώρες. Έτσι, η Gen-Probe οδηγεί σε γρήγορη και έγκαιρη διάγνωση της φυματίωσης συμβάλλοντας: α) στην άμεση έναρξη της θεραπείας με αντιφυματικά φάρμακα και β) στη μείωση των εξόδων νοσηλείας στο νοσοκομείο.

Αιτίες Κλειδιά: Φυματίωση, Μυκοβακτηρίδια, AIDS, HIV λοίμωξη, Ανιχνευτής.

REFERENCES

7. TB and HIV Coinfection, CDC NCHSTP Division of Tuberculosis Elimination.
10. Quality Performance Learning series: Tuberculosis case management. Quality Assurance Project. E-mail gapdissem@unc-chs.com
